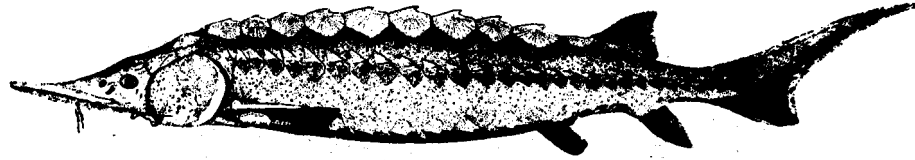


Atlantic sturgeon

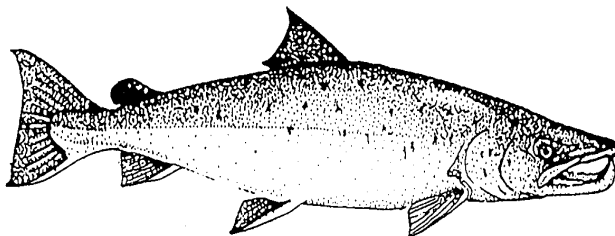


Annual Report of Biological Activities

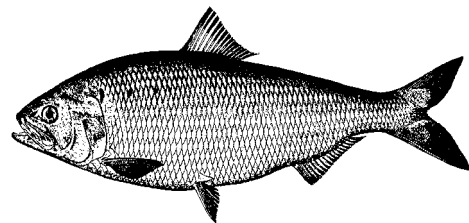
* 1999 *

U.S. Fish and Wildlife Service

Northeast Fishery Center
Lamar, Pennsylvania
(570) 726-4247



Atlantic salmon



American shad

STUDIES PERFORMED.- Fiscal year 1999 challenged the staff of the Northeast Fishery Center (NEFC) with some new and interesting problems. The pool of diverse talents represented by our staff was expanded with the addition of population ecologist and biometrician, Dr. Michael Millard. Targeted species for applied research this year were: Atlantic salmon, Atlantic sturgeon, American shad, striped bass, and rainbow trout. NEFC actively participated on an international basis in a number of arenas including: (1) European symposium to save the globally endangered European Atlantic sturgeon *Acipenser sturio*, (2) 1999 International Convention for Exploration of the Seas (ICES) Annual Science Conference, and (3) the U.S. Atlantic Salmon Assessment Committee, the technical body which responds to the North Atlantic Salmon Conservation Organization (NASCO).

Study Number and Title:

(Previously unreported results from 1998 experiments):

- LM-98-01 Effect of two broodstock diets upon rainbow trout reproductive success

- LM-98-09 Effect of density on mortality of green and eyed Atlantic salmon eggs and size of alevins in vertically-stacked incubator trays at White River National Fish Hatchery (Phase II)

(Current fiscal year, 1999 studies):

- LM-99-01 Investigations relative to poor reproductive success of landlocked Atlantic salmon *Salmo salar* reared at Allegheny Nation Fish Hatchery - incubation density and cold water disease.

- LM-99-02 Evaluating survival of hatchery-origin Atlantic salmon fry in the W. Branch Pleasant River, Penobscot Drainage, Maine.

- LM-99-03 An evaluation of growth and feed efficiency of sub-yearling Atlantic sturgeon *Acipenser oxyrinchus* at different feed rates.

- LM-99-04 Use of calcein as an alternative or in combination with oxytetracycline for increased flexibility in batch-marking larval American shad *Alosa sapidissima*.

- LM-99-05 Growth and survival of hatchery-reared Atlantic sturgeon at various rearing densities.

- LM-99-06 Use of fast balneation techniques to induce a calcein mark in Atlantic salmon sac-fry

OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:

- LM99A Mortality associated with catch and release of American shad and striped bass on the Hudson River
- LM99B Development of prototype hand-held calcein detection device
- LM99C Tank spawning of American shad using time-released hormone implants
- LM99D An automated laboratory to study the influence of various dissolved oxygen regimes upon fish
- LM99E Fish Health Inspection/Monitoring/Diagnostic Services
- LM99F Participation in the National Wild Fish Health Survey
- LM99G Cooperative work on a newly found virus of Atlantic salmon
- LM99H Participation in Maine Fish Health Advisory Board concerning ISAv Issues

STUDIES IN WHICH THE CENTER COOPERATED:

Osmotic induction of calcein into emergent chum salmon in Alaska and mark detection using a hand-held device. - *Jim Finn, U.S. Geological Survey, Biological Resources Division, AK.*

Cryo-preservation of Atlantic sturgeon milt. - *William Wayman, U.S. Fish and Wildlife Service, Warm Springs Fish Technology Center, GA.*

Investigating optimum fry stocking levels for Atlantic salmon in New England waters. - *Jan Rowan, Chair; U.S. Atlantic Salmon Assessment Committee, Nashua, NH.*

Development of a statistically robust spawner survey for horseshoe crabs in Delaware Bay. - *Dr. Dave Smith, USGS, Leetown Aquatic Ecology Laboratory, WV.*

Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast. Report to the Atlantic States Marine Fisheries Commission horseshoe crab technical committee. August 1998. - *Tom O'Connell, Horseshoe Crab Management Plan Coordinator, Baltimore MD .*

Gillnet selectivity for the spring weakfish fishery near Delaware. *Gary Swihart, USFWS, Gloucester Fisheries Assistance Office, Gloucester VA.*

Review and compilation of statutes, policies, and data relevant to the restoration of lake trout in the upper Great Lakes.- *Brian Lubinski, Team Leader, USFWS Region 3 Fisheries Program, Minneapolis, MN.*

PUBLICATIONS:

Mohler, J.W. and J.W. Fletcher. 1999. Induced spermiation in wild Atlantic sturgeons held captive up to six years. *North American Journal of Aquaculture*. 61:70-73

Mohler, J.W., M.K. King, and P.R. Farrell. 2000. Growth and survival of first-feeding and fingerling Atlantic sturgeon under culture conditions. *North American Journal of Aquaculture*. In progress.

TECHNICAL INFORMATION LEAFLETS:

LM-97-04/98-09 Effect of density on Atlantic salmon eggs and alevins in incubator trays at White River National Fish Hatchery - Bethel, VT (*Phase I and Phase II studies*)

FORMAL PRESENTATIONS:

Barbash, P. - Poor Reproductive Success of Landlocked Atlantic Salmon at Allegheny National Fish Hatchery. 24th Annual Eastern Fish Health Workshop. March 8-11, Atlantic Beach, North Carolina.

Barbash, P. -A Demonstration of the National Wild Fish Health Survey Database - Internet Accessibility and Applications. Annual Meeting of the New England Salmonid Health Committee. August 11-12, Concord, New Hampshire.

Barbash, P. -A Demonstration of the National Wild Fish Health Survey Database - Internet Accessibility and Applications. Atlantic International Chapter of the American Fisheries Society Annual Meeting. September 19-21, Campden, Maine.

Coll, J. -First Report of Swim Bladder Fibrosarcoma in Atlantic Salmon in the United States and an Associated Retrovirus. 24th Annual Eastern Fish Health Workshop. March 8-11, Atlantic Beach, North Carolina.

Coll, J. -Fish Health Impacts Between Feral and Cultured Fish. Atlantic International Chapter of the American Fisheries Society Annual Meeting. September 19-21, Campden, Maine.

Fletcher, John W. - An automated laboratory to study the influence of various dissolved oxygen regimes upon fish. 55th Annual Northeast Fish and Wildlife Conference. April 11-14, Manchester, New Hampshire

Jodun, Wade. - Interaction between time and concentration of iodophor disinfections and effects on percent eye-up of Atlantic salmon *Salmo salar* eggs. 55th Annual Northeast Fish and Wildlife Conference. April 11-14, Manchester, New Hampshire

PRESENTATIONS (continued)

King, M. Kim. - LC50 determination for three therapeutic chemicals on Atlantic sturgeon *Acipenser oxyrinchus* fingerlings. Fisheries Poster. 55th Annual Northeast Fish and Wildlife Conference. April 11-14, Manchester, New Hampshire

Millard, Michael J. The stock assessment process for the horseshoe crab; searching for clues. . American Fisheries Society Annual Meeting. August 31 Charlotte, North Carolina.

Millard, Michael J. The horseshoe crab stock assessment process: status and update on the resource in coastal bays. Delmarva Coastal Bays Conference III. November 12, Ocean City, MD.

Mohler, Jerre W. Early culture of Atlantic sturgeons *Acipenser oxyrinchus* for restoration stocking or aquaculture. Symposium on Conservation of the Atlantic sturgeon *Acipenser sturio* in Europe. September 6 - 11, Madrid and Sevilla, Spain.

Mohler, Jerre W. - Growth and survival of first-feeding and fingerling-sized Atlantic sturgeons *Acipenser oxyrinchus* reared at different densities. 55th Annual Northeast Fish and Wildlife Conference. April 11-14, Manchester, New Hampshire

Mohler, Jerre W. - Short-term marking of American eel *Anguilla rostrata* elvers with the fluorescent compound, calcein. April 11-14, 55th Annual Northeast Fish and Wildlife Conference. Manchester, New Hampshire

Study Number: LM-98-01

Title: Effect of two broodstock diets upon rainbow trout reproductive success

Investigators: Bill Fletcher - Northeast Fishery Center (NEFC) and Dale Honeyfield - Biological Resources Division (BRD), Wellsboro and Co-investigators - Mike Hendrix and Jerre Mohler - NEFC; Bill Krise - BRD, Wellsboro; Kari Duncan - White Sulphur Springs NFH

Background and Justification

The mission of Ennis, Erwin, and White Sulphur Springs (WSS) National Fish Hatcheries is to produce adequate numbers of disease-free, genetically distinct strains of trout eggs to support the National Fish Hatchery System as well as other federal agencies, researchers, and cooperators.

Erwin strain rainbow trout at Ennis and White Sulphur Springs NFH have shown poor eye-up (about 75%) for a number of years. Although many environmental variables are present, diet formulation can be examined without major modification of facility operations and has been demonstrated to impact egg and fry quality.

Study Objectives

The objective is to determine the effect of diet on reproductive performance of about 600 WSS NFH rainbow trout (Erwin strain) broodstock fed the current standard pellet diet (GR7-30, double vitamin pack) or a nutritionally updated extruded diet (RBT-5) in 1998.

Methods

Diet.- The experimental RBT-5 diet was prepared using modern extrusion technology which has been shown to improve nutrient availability including addition of ingredients which may effect reproductive performance. The mineral supplements for the RBT-5 diet, was provided in an organic matrix, metal - proteinate form, which are biologically available at an increased level, compared to formulations used in GR 7-30. The RBT-5 diet contained a vitamin premix at 2.5 times more than listed requirements including vitamin C in a protected form at eight times the NRC recommended level of 50 mg/kg. Perdue Feed Inc. produced the diets.

Culture.- The study commenced at WSS NFH in Feb. 1998, to provide a five month minimum diet period. Four raceways containing a total of 4,000 Erwin strain rainbow trout were used in the study. A total of 600 were Floy-tagged and measured at start and just prior to spawning. Two raceways each were fed either RBT-5 diet or GR7-30 diet.

Spawn. - A total of 120 spawns were evaluated, 60 from each diet treatment. Each spawn was enumerated and incubated in partitioned Heath trays. Egg samples each group were collected prior to fertilization and frozen for nutrient analysis.

Results

Data analysis is not finalized

Study Number: LM-98-09

Title: Effect of density on mortality of green and eyed Atlantic salmon eggs and size of alevins in vertically-stacked incubator trays at White River NFH (Phase II)

Principal Investigator: Jerre W. Mohler-Northeast Fishery Center-Lamar, PA (NEFC)

Co-investigators: Ken Gillette and Bruce Jensen-WRNFH; Michael Millard-NEFC

Background and Justification:

In 1997, Phase I of this investigation was performed with eggs of domestic Connecticut River Atlantic salmon (ATS) at White River National Fish Hatchery (WRNFH). A large proportion of eggs produced in Region 5 salmon hatcheries are incubated there due to favorable water temperatures and facilities. In some years it is necessary to optimize use of existing incubation facilities. This can be accomplished by incubating more eggs per tray but effects of elevated egg densities on egg mortality and alevin size are not known for this species. In 1997, we tested green eggs at 8,000 - 12,000 per tray (eyed-eggs at 6,500 - 10,500 per tray) and found that percent mortality, blue sac, and deformed fry were not different between treatments. Furthermore, alevins were larger at the elevated densities than those incubated at normal production levels. This study explored egg densities on a production scale at WRNFH.

Study Objectives

We compared effects of two egg densities on mortality and alevin size using 660,000 green and 570,000 eyed Atlantic salmon eggs at WRNFH during the 1998/99 incubation period. Additionally, we compared effects of egg density on mortality and alevin size with 14,000 green/12,000 eyed per tray. Finally we compared effects of three egg densities on mortality and alevin size of an undetermined number of kelt eggs. Recommended maximum egg densities for ATS eggs at WRNFH will result.

Materials and Methods

Experiment #1.-660,000 pooled eggs from domestic ATS were placed into four full stacks of incubator trays. Two stacks contained 10,000 and two contained 12,000 eggs/tray. **Experiment #2.**- 316,000 fertilized eggs were composited and placed into trays at three densities (10,000 - 12,000 - 14,000). Each treatment had two replicates at each of three tray levels (high, medium, low). The balance of trays throughout the stacks were stocked at densities normally maintained at WRNFH. **Experiment #3.**- We composited 20,600 mls of kelt eggs from N. Attleboro NFH and established three egg densities: 910, 1140, and 1380 mls per tray (based on egg volumes tested in study number L-97-04). The balance of trays throughout stacks contained kelt eggs at densities normally maintained at WRNFH. At eye-up, mortality was compared and eggs were composited within their experimental densities and redistributed into trays at reduced densities commonly used at WRNFH. Alevins were held in trays and inventoried prior to stock-out.

Results

Experiment #1:

- a) There was no difference in eye-up or fry mortality between 10K and 12K treatments.
- b) Average weight of fry was similar between densities, but a trend for smaller fry with decreasing tray position was seen at both densities.

Experiment#2:

- a) High position trays had greater eye up (94.3%) than others (89.9%)
- b) Eggs incubated at 14K had lower eye-up (87.9%) than 10K (92.3%) and 12K at (93.7%)
- c) Tray position effected fry mortality at all densities tested - middle trays had least mortality (2.1% vs. 6.6% at low trays). - Fry in middle trays were somewhat larger than others.

Experiment #3:

- a) Eye-up of kelt eggs was lower (69%) at the high density vs low density (72.2%)
- b) Eye-up was lower at low tray positions (63.3%) vs mid and high positions (73.9 and 75.1%).
- c) Fry mortality was not different between all densities tested.
- d) Fry mortality was higher at low tray position (14.8%) vs high position (12.2%)
- e) Fry incubated at 1140 ml/tray had higher average weights than their study counterparts.

Study Number: LM-99-01

Title: Investigations relative to poor reproductive success of landlocked Atlantic salmon (*Salmo salar*) reared at Allegheny National Fish Hatchery -incubation density and cold water disease

Principal Investigators: Bill Fletcher ; Trish Barbash,- Northeast Fishery Center (NEFC)

Co-Investigators: Fred Griffiths - Allegheny National Fish Hatchery (A.LL NFH) ; Jerre Mohler (NEFC)

Background and Justification:

Fish production programs at Allegheny NFH are directed toward restoration stocking of lake trout for the Great Lakes and production of 200,000 feeding Landlocked Atlantic salmon (LAS) fry for Lake Ontario. Recently, there has been a trend of poor survival of LAS broodstock and poor reproductive success. Only 8 percent of West Grand Lake LAS spawn in 1996 and 6 percent in 1997 survived to stocking. Initial eye-up was low and variable - 10 to 45% in 1996 and 4 to 73% in 1997. Hatchery protocol has included dry spawning of eggs, water hardening in iodophor, followed by loading Heath incubators at three quarts (2835 ml) green eggs per tray and two quarts (1890 ml) eyed eggs per tray. Infestations of *Saprolegnia* sp. have been precluded by prophylactic treatments of Paracide F. It has been noted that the eggs were soft and very fragile. Two strains of LAS broodstock will be used this year; West Grand Lake (GLW), now 6 years of age and 4-year-old (first time spawners) Sebago Lake (SEB) .

The present study will examine two potential factors which may affect salmonid reproductive success - incubation density and the potential impact of the causative agent of Coldwater disease, *Flavobacterium psychrophilum*. Work will be conducted at two sites. Currently female LAS number approximately 40 GLW and 360 SEB at Allegheny NFH and 8 GLW females at NEFC.

Study Objectives:

The objective of the present study is to test the following null hypotheses: (1) Reduction of green and eyed egg incubation loading densities will not impact egg or fry survival in LAS at Allegheny NFH (2) The pathogen *Flavobacterium psychrophilum* is not present in LAS at ALL NFH or if present its abundance is not impacted by egg densities.

Methods:

Three lots of LAS eggs will be tested for response to Heath tray density loading and occurrence of *E. psychrophilum* - Sebago strain at ALL NFH and Grand lake strain at NEFC and ALL NFH. Milt with verified motility will be collected from 10 to 12 males. Eggs from about 8 females (~ 36,000 in each group) will be fertilized using the dry method in a 2 male :1 female ratio. Egg water-hardening and disinfection in iodophor will follow Service Fish Health Guidelines; eggs will be water-hardened in 50 mg/L iodine solution for 30 minutes. Divided trays will be used to separate study lots. Once water hardened, eggs will be composited and thoroughly mixed. Initial pick of dead eggs will be conducted if feasible. Three replicates for each treatments will be randomly assigned to divided trays in proportions equivalent to full tray densities of 96 oz, 72 oz, and 48 oz (2835 ml, 2126 ml, 1418 ml). Paracide F will be used to control *Saprolegnia* sp. . Following enumeration of eyed eggs, compositing and mixing will be repeated and eggs will be assigned proportionally to full tray equivalents of 64 oz, 48 oz, and 32 oz (1890 ml, 1418 ml, 945 ml) . Survival to button up fry for each unit will be recorded. Bacterial screening for *F. psychrophilum* will be conducted for each replicate at eyed egg and sac fry stages.

Results:

The study is on-going

Study Number: LM-99-02

Title: Evaluating survival of hatchery-origin Atlantic salmon fry in the W. Branch Pleasant River, Penobscot Drainage, Maine.

Principal Investigator:- Mike Millard, Northeast Fishery Center (NEFC)

Co-Investigators: Bill Krise USGS/BRD -Wellsboro, PA; Tracey Copeland and Dan Kircheis - Maine Fisheries Coordinator, East Orland, ME

This study was not performed due to scheduling conflicts in the Maine Fisheries Coordinators Office.

Study Number: LM-99-03

Title: An evaluation of growth and feed efficiency of sub-yearling Atlantic sturgeon *Acipenser oxyrinchus* at different feed rates.

Principal Investigator:- Wade Jodun, Northeast Fishery Center (NEFC)

Co-Investigators: - Kim King, Pat Farrell, Mike Millard (NEFC)

Background/Justification:

With many sturgeon species teetering on the verge of extinction, the U.S. Fish and Wildlife Service has also intensified efforts to reestablish the fish. In fact, the Service has adopted "The Framework for the Management and Conservation of Paddlefish and Sturgeon Species in the United States". This guideline also calls for aquaculture research to evaluate the potential for wild population enhancement through the release of cultured fish. The 1998 Status Review for Atlantic Sturgeon also encouraged "the continuation and refinement of basic culture experiments to provide information on nutritional requirements and feeding techniques" for the species. Therefore, the Northeast Fishery Center in Lamar, PA has undertaken the challenge of developing the technology necessary to advance and refine Atlantic sturgeon culture. Since 1993, numerous culture efforts have strengthened our knowledge of Atlantic sturgeon and advanced growth and survival of the species under hatchery conditions. However, according to Mohler et al. 1996, additional study is needed to define the dietary requirements for accepted growth of cultured Atlantic sturgeon. McPeck and Arnold (1999) investigated the relationship of feed rate and water temperature to growth and survival of Atlantic sturgeon, but in general little is known about optimum feeding rates for Atlantic sturgeon.

Study Objectives:

The primary objective of this study is to discover the optimum feed rate, expressed as percent body weight per day (BWD) for maximum survival, growth and feed conversion of sub-yearling Atlantic sturgeon (mean initial weight = 28 grams).

Materials and Methods:

Sub-yearlings produced at NEFC from Hudson River broodstock will be used for the study. One hundred-fifty sturgeon will be pooled and randomly distributed into six 4-foot-diameter circular tanks at a density of 25 fish/tank. Initial biomass tanks will be adjusted to within $\pm 5\%$ of each other. Two replicates for each of three feeding rates (1.5, 3.0, or 4.5% BWD) will be randomly assigned to tanks. Feed (Zeigler Brothers, Inc. sturgeon diet, Gardners, PA) will be delivered by way of automatic feeders modified to dispense feed continuously over 24 hours. Tanks will be cleaned daily and feed amounts, mortality, water temperature, and dissolved oxygen will be recorded on a daily basis. Weekly inventories will be performed on individual fish and feed rates will be adjusted accordingly. The study will continue for 45 - 60 days when individual fish will undergo final inventory.

Results:

- 1) Even though growth was greatest at the 3.0% feed rate, sturgeon were more efficient at conversion of food to flesh at the low (1.5%) feed rate.
- 2) Weekly biomass increases for fish at 1.5, 3.0, and 4.5% feed rates were 16.4, 20.5, and 16.5%, respectively.
- 3) Feed Conversion Rates for fish at 1.5, 3.0, and 4.5% feed rates were 0.64, 1.09, and 1.88, respectively.

Study Number: LM-99-04

Project Title: Use of calcein as an alternative or in combination with oxytetracycline for increased flexibility in batch-marking larval American shad *Alosa sapidissima*

Principal Investigator: Jerre Mohler (Northeast Fishery Center)

Co-Investigators/Cooperators: John Fletcher, Michael Millard (Northeast Fishery Center)

Background and Justification:

Restoration of American shad is a high priority for federal and state fisheries agencies in the northeast and mid-Atlantic regions of the U.S. The state of Pennsylvania has been involved in shad restoration on the Susquehanna River since the early 1980's and has received an average of 32.7 million eggs per year since 1982 from the states of New York and Delaware for use in the program. The states of Virginia and Maryland are also currently active in restoration of many Chesapeake Bay tributaries such as the Susquehanna, Potomac, James, Chester, and Naticoke Rivers. The state of Maine plants about 2 million shad fry annually in the Kennebec River, and ½ million annually in each of the Sebasticook and Medomak Rivers. Shad incubated in these programs are stocked as non-feeding fry and receive a chemical immersion bath in oxytetracycline at about 250 mg/L at various daily intervals to produce a series of otolith marks according to stocking location. When stocked fry return as spawning adults, otolith samples are taken in order to assess the stocking program. Since 1995, NEFC has experimented with calcein to produce a non-lethally detectable mark in fin tissue of Atlantic salmon, and found that the compound marks otoliths as well. If calcein produces a similar mark in shad, the prospect of combining calcein and oxytetracycline marks in the same fish would permit greater marking flexibility.

Study Objectives:

This study will assess otolith marks induced by immersing 5000 non-feeding shad fry in calcein and/or oxytetracycline in the spring of 1999 with annual mark reassessment to sexual maturity.

Materials and Methods:

About 5000 shad fry produced from tank spawning adults at NEFC will be used in the study.

Immersion will be performed as follows:

<u># Fry</u>	<u>Treatment</u>	<u>Duration</u>	<u>Interval</u>
1000	Oxytet. @256 mg/L	4 hours	3, 6, and 9 day post-hatch
1000	Calcein @250 mg/L	4 hours	3, 6, and 9 day post-hatch
1000	Controls	4 hours	3, 6, and 9 day post-hatch

Combined marks:

1000	Oxytet. @ 256 mg/L on day 3 - Calcein @250 mg/L on day 9
1000	Calcein @ 250 mg/L on day 3 - Oxytet. @256 mg/L on day 9

Fry will be treated in triplicate in 2-ft diameter tanks. After immersion schedules are completed, 20 fry from each treatment will be analyzed for mark readability using fluorescence microscopy. At least 20 fish will be sacrificed annually for mark assessment until fish are sexually mature.

Results:

Twenty-four-hour mortality was high in all treatments (92% for both oxytetracycline and calcein-exposed fish and 59% for controls). Sub-samples of survivors showed that larvae in both chemical treatments received a uniquely discernible otolith mark. Oxytetracycline-marked otoliths were observed as a pale-yellow fluorescence while those marked with calcein were best described as emitting an apple-green fluorescence. Fluorescence of oxytetracycline-marked otoliths tended to fade with exposure to light during microscopic examination therefore photomicrographs were too faint to develop. Subsequent immersions intended to induce multiple and differential marks using both chemicals were not possible due to nearly complete mortality in all treatments (including controls) by 48-h after the first immersion bath. Mortality was likely due to excessive handling of fry.

Study Number: LM-99-05

Title: Growth and survival of hatchery-reared Atlantic sturgeon at various rearing densities

Principle Investigator: Wade Jodun, Northeast Fishery Center

Co-investigators/Cooperators: Jerre Mohler, Michael Hendrix, John W. Fletcher and Michael Millard; Northeast Fishery Center

Background/Justification:

The continued decline of Atlantic sturgeon across its historic range prompted the Atlantic States Marine Fisheries Commission (ASMFC) to begin the development of a Fishery Management Plan (FMP) in 1988. One of the plan's primary recommendations calls for an expanded aquaculture effort and the development of rearing techniques applicable to Atlantic sturgeon (Taub, 1990). In response to these recommendations, the U.S. Fish and Wildlife Service has also intensified efforts to reestablish the fish. The 1998 Status Review for Atlantic Sturgeon also encouraged "the continuation and refinement of basic culture experiments to provide information on optimal environmental rearing conditions" for captive propagation of the species. Numerous investigations by NEFC personnel have steadily advanced our knowledge of culture techniques for the species (Mohler et al., 1996; Mohler and Fletcher, 1998; Mohler et al. 2000). However, information pertaining to rearing densities for year-class Atlantic sturgeon and other sturgeon species was not found. This investigation will examine the impact of densities on growth and survival of yearling Atlantic sturgeon.

Study Objectives:

The primary objectives of this study are to compare: (1) Specific Growth Rate (2) Feed Conversion and (3) survival of 300 yearling Atlantic sturgeon reared at various densities over a period of 4 to 6 weeks beginning in August 1999.

Materials and Methods:

Thirteen-month-old Atlantic sturgeon spawned using gametes from one female and five males captured from Hudson River in 1998 will be used for the study. Three hundred sturgeon will be pooled and randomly distributed into fifteen 4-foot diameter, circular tanks at stocking densities ranging from 3.59 - 10.85 kg/m² (or 7.95 - 24.06 g/L). Three replicates of each density will be established with equivalent biomass. Flow rate will be 16 L/minute at ambient temperature. Zeigler Brothers, Inc. sturgeon diet (Gardners, PA) will be offered at 2.5% tank biomass per day via automatic feeders modified to dispense feed continuously over 24 hours. Tanks will be cleaned daily and mortalities, water temperature, and dissolved oxygen will be recorded daily. Weekly inventories of individual fish will be performed and feed rates will be adjusted accordingly. Rearing will continue for 30 - 60 days or until distinguishable differences in growth can be observed. At the conclusion of the study all fish will be individually inventoried to determine final survival and growth.

Results:

After 49 days, fish from the lowest density treatment had greater mean weight (673.5 g) than those maintained at the highest density (560.7 g). Likewise, body weight increase was 101.0% for fish in the lowest density and 54.7% for those in the highest density. Feed Conversion Rates ranged from 1.93 - 2.94 with fish at the lowest density being more efficient at converting feed to flesh than those at the highest density. However, Feed Conversion Rates were not at maximum levels for this species since feed was offered in excess to ensure that all treatments were fed to satiation.

Study Number: LM-99-06

Title: Use of fast balneation (osmotic induction) techniques to induce a calcein mark in Atlantic salmon sac-fry

Principal Investigator: Jerre W. Mohler - NEFC

Background and Justification:

Using fry plants as a primary management tool for Atlantic salmon restoration by the New England states and the U.S. Fish and Wildlife Service appears to be a successful strategy based upon the numbers of adult fish which returned to the Connecticut River in 1998. As this strategy becomes an increasingly important part of the Atlantic salmon (ATS) restoration program, the need for fry marking techniques also increases. Since 1995, NEFC biologists have been testing calcein as an immersion-marking chemical on larval ATS for effects on short and long term mark retention, health, and growth. Fish immersed in calcein solution for 24 hours receive marks detectable as brilliant green fluorescence in all fin ray structures when viewed using fluorescence microscopy. Marks have been non-lethally detected in 58 out of 61 (95%) sampled at 8 months post-immersion. Marks have also been detected at 22 months post-immersion. Previous trials showed that calcein mark uptake can be variable, depending on chemical concentration, immersion time, and temperature. Therefore, it is necessary to discover techniques which reduce immersion times yet still yield consistently bright and long-lasting marks. In 1998, NEFC successfully marked American eel elvers with calcein using a 3.5-minute, 3% salt bath followed by immersion in 1 - 2% calcein solutions for 3.5 minutes. We propose to explore this same "osmotic shock" technique on Atlantic salmon sac-fry.

Study Objectives:

We will use a combination of salt baths followed by immersion in concentrated calcein solutions to rapidly induce a calcein mark in caudal fin tissue in February 1999 ATS sac-fry. Thirty-day growth and mortality will be compared to controls on the set of treatments which show at least 90% survival along with consistent, brightly fluorescing marks in caudal fin tissue.

Materials and Methods:

Immersion trials will consist of calcein static baths with 3 replicates in 6 .5-liter jars each containing non-feeding ATS fry of Connecticut River domestic origin. Salinity tolerance (3.5 minutes) of 30 fry per jar will be tested at levels ranging from 0.5 - 5.0%. Once salinity tolerances are determined, this information will be used to set up the osmotic shock or osmotic induction of calcein at concentrations ranging from 1.0 - 2.0%. In addition, three control replicates will receive salt baths but will not be exposed to calcein. Those fish not sacrificed for calcein mark determination will be transferred into rearing tanks for 30-d growth and mortality assessment. Two repetitions of each treatment will be established with 30 fish each. A supply of live *Artemia* along with formulated salmon starter will be introduced to each replicate daily. Calcein chemicals are not FDA-approved for fish use, therefore exposed fish will never be consumed or released into the wild but will be held captive at NEFC for a length of time deemed necessary by NEFC biologists, then destroyed.

Results:*Salinity tolerance:*

- 1) Fish which received 5% salt for 3.5 minutes had greater 5-day survival than all others (including controls) suggesting that salt had some therapeutic effect.
- 2) Behavior of fish did not appear to change when exposed to experimental salinities (0.5 - 5.0%)

Calcein mark induction:

- 3) Immersion in at least 1% salinity for 3.5 minutes followed by a 3.5-min immersion in at least 1% calcein was required for consistent, brilliant mark induction.
- 4) Long-term growth and survival was not assessed due to disease outbreak and complete mortality in all treatments

OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:

LM99A Mortality associated with catch and release of American shad and striped bass on the

Hudson River.- Catch and release fishing has increased in many fisheries, such as the striped bass) and American shad fisheries of the Atlantic Coast, yet its contribution to fishing mortality is often underestimated. Recent striped bass reports indicate that anglers release over 90% of their catch. There is also evidence to suggest that American shad are intolerant to handling. Consequently, hooking mortality may contribute substantially to fishing mortality in the Atlantic striped bass and American shad fisheries. Hooking mortality of American shad was assessed by angling with light action spinning gear in the Hudson River immediately downstream of the navigation lock and dam at Troy, New York. Striped bass were caught with live bait (herring) on spinning gear near the mouth of Catskill Creek at Catskill, New York. Volunteer recreational fisherman were used for both species. After being angled, fish were transported and placed into holding pens anchored in the river. Control fish were captured by electroshocking and were transported and placed in the holding pens similarly to treatment fish. Fish were held in the pens for 5 days, after which time fish were measured and survival was recorded.

Results showed that a significant amount of mortality is associated with the hook and release of American shad. Both of two estimation techniques estimated approximately 30% mortality after five days. The estimated hooking mortalities for striped bass was 30.7% . When making the adjustment assuming additive finite rates, the estimated mortalities associated with hook and release for striped bass was 28.3%

LM99B Development of prototype hand-held calcein detection device.- As part of the continuing development of fry marking and mark-detection techniques, NEFC has completed a U.S. Dept. of Interior Report of Invention form for a potential patent concerning both a bench-top and hand-held calcein detection device which will make it feasible to quickly and efficiently detect fluorescent marks on individual fish under rigorous field conditions without the need for a microscope.

LM99C Tank spawning of American shad using time-released hormone implants. - In a continuing cooperative effort with partners in the Susquehanna River Anadromous Fish Restoration Committee, we experimented with tank spawning technology for American shad with the ultimate goal of using this technology to assist in establishing self-sustaining populations to the West Branch of the Susquehanna River. Work modifications from the previous year included: (1) increasing hormone (LHRHa) dosage to enhance egg production (2) development of a submergible egg separator to facilitate handling, grading, and cleaning eggs (3) manipulating incubation temperature and light, and (4) use of white fry tanks with dark-colored center drains (to facilitate cleaning and removal of fry from tanks). Other ideas tested included: (1) comparison of egg production in green vs. advanced brood (2) holding of immature brood for later hormone injection, and (3) investigation of the impact of varying LHRHa implant dosage. From testing these ideas we found no difference in egg production in immature vs. advanced brood, minimal advancement in egg development when holding immature brood, and no difference in egg production when LHRHa dosage was varied. In addition, we found that holding injected fish resulted in stress-induced mortality.

This year, production efforts were impaired due to immature female brood with poor quality eggs and the possibility of an inappropriate carrier substance for the spawning hormone. Additionally, fish were under excessive stress due to holding, transport, and handling conditions which resulted in high mortality in our tank spawning system. A total of 364 female and 588 male AMS were transported to the NEFC from April 27 to June 5. A total of 412,613 eggs were collected and incubated resulting in a hatch of 17,950 fry which were used in a study to assess the viability of alternate fry tagging techniques. Results of the FY99 AMS spawning season have led to changes in our focus for the American shad program. In FY2000 less

emphasis will be placed on quantity of egg production and more on improving tank spawning techniques, testing alternative marking techniques, and mitigating handling stress.

LM99D An automated laboratory to study the influence of various dissolved oxygen regimes upon fish. - Many hydroelectric plants rely upon hypo-limnetic discharges from reservoirs which may introduce poorly oxygenated waters into receiving streams during power generation. We developed NITROS (nitrogen injection to regulate oxygen saturation), a dissolved gases laboratory, to test affects of various oxygen regimes upon fish growth. NITROS system components interact through monitoring feedback loops to record data, operate alarm and back-up systems, and to automatically stabilize or change supply water temperatures and dissolved oxygen levels in sixteen fish culture tanks. Tempered water is degassed to near saturation levels by packed columns and in the next phase, nitrogen gas is injected into negative pressure chambers to displace oxygen to obtain required dissolved oxygen (DO) swings. Treated water is then pumped to individual culture tanks. Temperature and oxygen probes provide variable current signals to PID type digital controllers which in turn operate either tempered water mixing valves or linearly proportional nitrogen gas valves to maintain selected parameters. An initial 12-week growth trial with rainbow trout was conducted with four DO regimes which simulated different hydroelectric discharge patterns. Desired temperature was maintained $\pm 1^\circ\text{F}$ and DO levels were routinely kept at $\pm 0.3\text{ mg/l}$ of targets. With long term study capabilities, the NITROS laboratory may be used to investigate affects of periodic low DO discharges and temperature fluctuations upon fish growth and other physiological responses. Additionally, the automated feedback design of NITROS has the potential to utilize remote sensors to establish realtime set points of actual field environmental conditions for testing.

LM99D Fish Health Inspection/Monitoring/Diagnostic Services. - The Lamar Fish Health Unit processed 365 laboratory cases in fiscal year 1999. Region 5 has a very extensive fish health monitoring program to enhance the fish health inspections, allowing continual surveillance of the health status of the stocks, some of which have been identified as very limited distinct population segments (DPS) which the Service has just recently proposed for listing under the Endangered Species Act (ESA). The Fish Health Unit had 32 inspection cases, which included 13 that were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These statistically based fish health examinations are essential to prevent the spread of fish diseases through fish and/or egg transfers and are necessary to enable facilities to comply with regulations on transporting and releasing fish. In addition to the 205 monitoring cases involving examination of fish, 4 Service facilities provided 31 water monitoring cases, where water from rearing units is examined by the water filtration method, a very effective proactive protocol for diagnosing furunculosis before an epizootic occurs.

LM99E Participation in the National Wild Fish Health Survey. - This project, launched in 1997, continues to involve all nine Service fish health centers nationwide incorporating standardized diagnostic techniques and data management methods to ensure comparability. In fiscal year 1999, the Fish Health Unit initiated 65 cases for the NWFHS, in which 3,255 fish from a total of 85 sites were examined and 1,140 fish representing 83 completed cases were entered into the NWFHS database, soon to be accessible to all via the Internet. Interesting finding in fiscal year 1999 include the detection of *Renibacterium salmoninarum*. from 5 of 18 sites sampled in waters of the state of Vermont and from seven of 10 rivers sampled in Virginia's Shenandoah National Park in FY98. The pathogen was confirmed at about a 20% prevalence level at these sites, from salmonid samples. It was also confirmed from blacknose dace at one site. These same dace samples were also found positive for IPNV. Outreach activities to increase awareness of the Survey included demonstrations of the database on the Internet to the New England Salmonid Health Committee in August 1999 and to the AFS/ Atlantic International Chapter at their annual meeting in Camden Maine in September 1999. The Internet demonstrations sparked great interest in all meeting participants, and enthusiasm for efforts finally being made here to compile data on pathogens in the wild.

LM99F Cooperative work on a newly found virus of Atlantic salmon. - In fiscal year 1999, work with the new retrovirus (salmon swimbladder sarcoma virus) discovered in Atlantic salmon continued. A non-lethal PCR test for detection of this virus developed by Cornell University was used for screening Atlantic salmon in the spring and fall of 1999 at Craig Brook NFH, in Maine, where it was found that additional salmon stocks were also positive, but no clinical signs have ever been observed there. Clinical signs, including irregular skin patches, swimbladder tumors, and mortalities, were only observed at one of three facilities, where the environment and the diet were unique. Without any additional disease signs in subsequent research or production environments, Rivers Postulates have yet to be fulfilled. Though evidence to date indicates the virus probably came from wild parr taken from the Downeast Rivers of Maine, much more is needed to be understood about the virus's virulence, contagion, range, or general epidemiology.

LM99G Participation in Maine Fish Health Advisory Board concerning ISAv Issues.- The Maine Fish Health Advisory Board serves as a scientific advisory board to the state Commissioners. The group, containing a representative from the Fish Health Unit, is very heavily involved with Infectious Salmon Anemia virus (ISAv), presently an exotic to the United States, but with a geographical proximity right up to the border, as well as other fish health issues related to private aquaculture and wild resources. The Service, at the Lamar Fish Health Unit, has established a monitoring program for all sea-run Atlantic salmon mortalities as has the Maine salmon industry initiated an intensive ISAv monitoring program. Due to the low numbers of the distinct population segments (DPS) of Atlantic salmon, it is vital that the impacts of this and other diseases are held to a minimum.